

CLAIMS

We claim:

1. A method for determining a haplotype of a subject comprising the steps of:
 - (a) diluting a nucleic acid sample from the subject into a single molecule dilution;
 - (b) amplifying the diluted single nucleotide dilution with at least two different primer pairs designed to amplify a region comprising at least two polymorphic sites in the nucleic acid template;
 - (c) genotyping the polymorphic sites in the single nucleic acid molecule; and
 - (d) determining the haplotype from the genotypes of at least the two polymorphic sites to obtain a haplotype for the subject.
2. The method of claim 1, further comprising repeating steps a-c at least three times from the same nucleic acid sample to obtain at least four genotype replicas from the same subject and thereafter comparing the at least four genotype replicas to determine the haplotype.
3. The method of claim 2, further comprising comparing the haplotype with a haplotype from a control or a database of haplotypes from controls to determine association of the haplotype with a biological trait.
4. The method of claim 1, wherein the polymorphism is a single nucleotide polymorphism.
5. The method of claim 1, wherein the polymorphism is a deletion, an insertion, a substitution or an inversion.
6. The method of claim 1, wherein the polymorphism is a combination of one or more markers selected from the group consisting of a single nucleotide polymorphism, deletion, an insertion, a substitution or an inversion.
7. The method of claims 1-6, wherein genotyping is performed using primer extension and mass spectrometric detection.
8. The method of claim 2, wherein 12-18 genotype replicas are produced.
9. A method of diagnosing a disease condition or disease susceptibility by determining a disease related haplotype in a subject comprising the steps of:
 - (a) diluting a nucleic acid sample from the subject into a single molecule dilution;
 - (b) amplifying the diluted single nucleotide dilution with at least two primer pairs designed to amplify a region comprising at least two polymorphic sites in the nucleic acid template;

- (c) genotyping the polymorphic sites in the single nucleic acid molecule;
 - (d) determining the haplotype from the genotype of at least two polymorphic sites to obtain a haplotype for the subject; and
 - (e) comparing the haplotype of the subject to known disease-associated haplotypes, wherein a match in the sample haplotype with a disease-associated haplotype indicates that the subject has the disease or that the subject is susceptible for the disease.
10. The method of claim 9, further comprising repeating steps a – c at least three times from the same nucleic acid sample to obtain at least four genotype replicas from the same subject and thereafter comparing the at least four genotype replicas to determine the haplotype.
11. The method of claim 10, wherein 12-18 replicas are produced.
12. A method of determining a haplotype of a subject comprising the steps of:
- (a) treating a nucleic acid sample from the subject with a composition that differentially affects an epigenetically modified nucleotide in the nucleic acid sample to effectively create polymorphisms based on the epigenetic modification;
 - (b) diluting the treated nucleic acid sample into a single copy dilution;
 - (c) amplifying the diluted nucleic acid sample using at least two different primer pairs;
 - (d) genotyping the amplified sample; and
 - (e) determining the haplotype of the subject from the genotyped sample.
13. The method of claim 12, further comprising repeating the steps b-d at least three times to obtain at least four genotype replicas from the same subject and thereafter determining a haplotype of the subject based on the genotype replicas.
14. The method of claim 13, wherein 12-18 replicas are produced.
15. The method of claim 12, wherein the epigenetically modified nucleotide is a methylated nucleotide.
16. The method of claim 15, wherein the nucleic acid sample is treated with bisulfite.
17. A method of determining a haplotype in a subject comprising the steps of:
- (a) digesting a nucleic acid sample from the subject with a methylation-sensitive restriction enzyme so that either unmethylated DNA or methylated DNA is left intact, depending on which enzyme is used;

- (b) diluting the digested nucleic acid sample to a single molecule concentration;
 - (c) amplifying the diluted and undiluted nucleic acid sample with at least two different primer pairs;
 - (d) genotyping the amplified samples; and
 - (e) determining a haplotype of a methylated nucleic acid wherein at least one polymorphic markers next to the methylation site, together with the methylation site, constitutes a haplotype.
18. The method of claim 17, further comprising repeating the steps b-d at least three times to obtain at least four genotype replicas from the same subject and thereafter determining a haplotype of the subject based on the genotype replicas.